HHS Public Access

Author manuscript

Semin Oncol. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

Semin Oncol. 2015 August ; 42(4): 549–561. doi:10.1053/j.seminoncol.2015.05.006.

Corresponding author: Craig L. Slingluff, Jr., M.D., Department of Surgery, University of Virginia, PO Box 800709, Charlottesville, VA 22908, 434 924-1730, Fax: 434 243-6844, cls8h@virginia.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Financial disclosures:

Joseph Obeid: none

Yinin Hu: none

Craig Slingluff: see table below:

Commercial interest	What I received	My role	When	Support provided to:	Scientific/ research area	relevant
Immatics	Consulting fee	Scientific Advisory Board member	Ongoing	University of Virginia	Cancer vaccines, using peptides	Yes
Polynoma	Consulting fee	Principal Investigator, for phase III trial of POL-103A	Ongoing	University of Virginia	Cancer vaccines, melanoma	Yes
UVA Licensing and Ventures Group	Royalties	Co-inventor on peptides for use in cancer vaccines, some licensed to GSK	Ongoing	Self	Peptide vaccines for cancer	Yes
Glaxo Smith Kline (GSK)	Research funding	Funding for clinical trial of MAGE-A3 vaccine trial Mel55 (NCT01425749)	Ongoing	University of Virginia	Cancer vaccines, vaccine adjuvants	Yes
3M	Resiquimod for use in clinical trial of melanoma vaccine	Principal investigator of Mel60 trial (funded by Melanoma Research Alliance)	Ongoing, planned	University of Virginia	Cancer vaccines	Yes
Merck	PD-1 antibody for use in investigator-initiated trial	Principal investigator of Mel64 trial	Ongoing	University of Virginia	Melanoma immunotherapy	Yes
Curetech	Consulting fee	Scientific advisory board member	Past	University of Virginia	Melanoma immunotherapy	No
Castle Biosciences	Honorarium – consulting fee	Scientific advisory board meeting fall 2014	Past	Self	Melanoma clinical care	No
Dermagra m, LLC	Ownership interest (< \$2,500) – but this is to be dissolved	Partner in LLC	Ongoing	Self	Skin cancer screening: total body photography	No

Vaccines, adjuvants and dendritic cell activators – Current Status and Future Challenges

Joseph M. Obeid, M.D. [research fellow],

Department of Surgery, University of Virginia, Charlottesville, VA

Yinin Hu, M.D. [research fellow], and

Department of Surgery, University of Virginia, Charlottesville, VA

Craig L. Slingluff Jr., M.D. [Joseph Helms Farrow Professor of Surgery]

Department of Surgery, University of Virginia, Charlottesville, VA; and Director, Human Immune Therapy Center; Director, Human Immune Therapy Center, University of Virginia, Cancer Center

Abstract

Cancer vaccines offer a low-toxicity approach to induce anticancer immune responses. They have shown promise for clinical benefit with one cancer vaccine approved in the U.S. for advanced prostate cancer. As other immune therapies are now clearly effective for treatment of advanced cancers of many histologies, there is renewed enthusiasm for optimizing cancer vaccines for use to prevent recurrence in early stage cancers and/or to combine with other immune therapies for therapy of advanced cancers. Future advancements in vaccine therapy will involve the identification and selection of effective antigen formulations, optimization of adjuvants, dendritic cell activation, and combination therapies. In this summary we present the current practice, the broad collection of challenges, and the promising future directions of vaccine therapy for cancer.

Introduction

The role of the immune system in cancer therapy is now well established. Immune-compromised patients are at higher risk of developing malignancies and increased lymphocytic infiltration in cancers is associated with improved survival¹⁻⁴. Checkpoint blockade therapy induces durable major cancer regressions by unleashing pre-existing antitumor immunity. Cancers that respond best to checkpoint blockade therapy are those with high frequencies of mutations and with preexisting T cell responses to cancer antigens. However, many cancers have low frequencies of mutations and low rates of spontaneous T cell responses. Thus, increased T cell responses to cancer antigens may improve patient outcomes. Cancer vaccines may improve antitumor immunity by expanding T cell responses to cancer antigens and have a relatively mild side effect profile. Effective cancer vaccines offer promise to improve cancer therapy in the following settings:

1. In combination with checkpoint blockade, by inducing (or expanding) T cell responses to cancer antigens in patients without significant spontaneous antitumor T cell responses. This may improve tumor control when checkpoint blockade is otherwise ineffective, as has been demonstrated in murine models⁵.

2. As monotherapy in the adjuvant setting, by inducing protective antitumor immunity for patients at high risk for recurrence, for whom the toxicity of more aggressive therapy may not be acceptable.
3. In combination with adoptive T cell therapy, as has been demonstrated to be effective in preclinical studies^{6,7}.
4. As monotherapy in advanced cancer, as with Sipuleucel-T (Provenge), which is the first FDA-approved cancer vaccine, and which has prolonged survival in patients with hormone-refractory prostate cancer⁸.

Cancer vaccines can induce immune responses to cancer antigens, with immune response rates as high as 100% and can be durable^{9,10}. However, clinical response rates with vaccines alone have typically been only in single digits¹¹. New findings suggest, though, that the promise of cancer vaccines may be realized with newer approaches and by combination immunotherapies. There have been promising new findings with a peptide vaccine combined with IL-2¹², with long peptide vaccines¹³, a Listerial vaccine for pancreatic cancer¹⁴, and a peptide vaccine targeting a shared mutated neoantigen in glioblastoma¹⁵. A partial summary of these and other vaccines^{16–28} in late-stage development is provided in Table 1. New approaches to modulate the tumor microenvironment also are introducing new concepts about creating vaccines *in situ* with a patient's own tumor. This article briefly summarizes these new findings and opportunities to augment the clinical efficacy of cancer vaccines.

To date, only one cancer vaccine has been approved: Sipuleucel-T has increased survival of patients with asymptomatic castration-resistant metastatic prostate cancer in two trials⁸. However, there are more than 350 open trials of cancer vaccines listed in ClinicalTrials.gov (September 2014). These trials are testing vaccines across a wide range of cancers, with large numbers in non-small cell lung cancer, melanoma, brain tumors, and prostate cancer (Figure 1). In addition, there is a range of vaccine strategies in terms of the form of antigen employed with the most common being peptide or protein, dendritic cells or DNA vaccines (Figure 1). As these data become available, they offer promise to advance our understanding of optimal cancer vaccine strategies.

Antigen classification

The selection of antigen(s) targeted with a cancer vaccine is critical to vaccine specificity and effectiveness. Cancer antigens can be considered in several categories, as shown in Figure 2. They may be shared among many tumors or be unique; they may be derived from mutated or non-mutated antigens. Antigens presented by class I major histocompatibility complex (MHC) molecules can induce a cytotoxic T lymphocytes (CTL) response. These antigens are typically short (8–11 amino acids). Epitopes for helper (CD4+) T cells, however, are typically longer peptides, which are presented by Class II MHC molecules. These may be incorporated in vaccines as peptides themselves, protein, DNA or RNA encoding the proteins/peptides, or cellular lysate formulations (Figure 2).

Many antigens recognized by tumor-reactive T cells are shared among patients' tumors. If therapeutic, these shared antigens may have broad applicability, with associated practical

advantages for regulatory approval and cost-effectiveness. Among the commonly reported categories of shared antigens include those derived from proteins expressed by other somatic cells, most of which are either 1) differentiation tissue proteins or 2) overexpressed or aberrantly expressed in cancer cells (examples specified in Figure 2).

1. Tissue differentiation antigens are the most homogenous among tumors of the same tissue origin. Targeting these antigens has been successful in multiple cancer settings, either with vaccines or with adoptive therapy. Expression of these antigens in normal tissue predisposes to tolerance and autoimmunity, lymphocytes infiltrating human melanomas do commonly recognize these antigens and can kill cancer cells expressing them. They may be considered as targets in cancers where the normal tissues or cells expressing those proteins can be lost without compromising patient survival^{29;30}; examples include cancers of the prostate, thyroid, and melanoma (Table 2).
2. Overexpressed or aberrantly expressed antigens include Her2/neu and epidermal growth factor receptor (EGFR), which have been targeted in breast cancer and multiple epithelial cancers, respectively, with antibodies trastuzumab and cetuximab, and can be therapeutic despite expression on normal tissues. Her2/neu peptides have also been targeted with vaccines, with induction of CD8⁺T cell responses to those peptides and with encouraging clinical outcomes. A classic aberrantly expressed normal protein is MUC-1, a heavily glycosylated protein expressed at the luminal surface of normal glandular cells (and thus not normally exposed to circulating blood lymphocytes). However, in many cancers, including breast cancer, MUC-1 is under-glycosylated and aberrantly expressed on nonluminal cell surfaces, rendering it accessible to recognition by antibodies and to T cells³¹.

Neoantigens

Other antigens represent “neoantigens”, where a neoantigen is “a newly expressed or acquired antigen.” In common usage, the term “neoantigens” refer to antigens arising *de novo* from a cancer-specific mutational event. However, we propose that a broader definition of neoantigens should apply to include all antigens that are not expressed on normal somatic cells but expressed on malignant cells. These include mutated neoantigens, cancer/germ cell antigens, and phosphopeptide antigens.

Mutated neoantigens

The classic neoantigen is one arising from a single-nucleotide polymorphism (SNP) or other mutation acquired in malignant cells, and absent from other normal cells. These may be the result of a mutation unique to a specific patient or one that is very common across a range of cancer patients, such as BRAF^{V600E/K}, c-Kit^{L576P}, or selected KRAS and NRAS mutations^{32–34}. Neo-antigens also include introns that were found to be translated due to aberrant splicing such as (gp100-intron 4, TRP-2-int2)³⁵.

Cancer/germ line antigens

These proteins are expressed on a range of cancers and usually are expressed more in advanced cancers than early-stage cancers. These antigens are also expressed during fetal development. In adults they are only expressed in developing gametes which normally do not express antigen-presenting MHC and are not accessible to the immune system³⁶. Therefore, T cell epitopes derived from these proteins are usually unique to tumor cells and present a low risk of autoimmunity and tolerance. They include MAGE-A1, MAGE-A3, GAGE, BAGE, RAGE, NY-ESO-1, and TAG-1 proteins³⁷. These antigens can be expressed in different cancers at variable levels of prevalence and can be targeted effectively³⁸. An experience with adoptive T cell therapy, however, raises concerns about unanticipated risks of toxicity from targeting these antigens. Two of five patients treated with adoptive transfer of cells transduced with a T cell receptor with high affinity for a MAGE-A3 peptide experienced lethal neurotoxicity attributed to cross-reactivity on MAGE-A12 which was found in the brain³⁹. Thus, cancer/germ line antigens may not always be expressed only on cancer cells or germ cells. Similar toxicity has not been reported with MAGE-A3 vaccines or other vaccines targeting cancer/germ line antigens. However as more aggressive combination immunotherapies subvert immune regulation that limits autoimmune toxicities, attention must be paid to such potential toxicities.

Phosphopeptide antigens

Phosphopeptides, presented by Class I or II MHC molecules, are derived from self-proteins whose phosphorylation is related to oncogenic activation. T cells reactive to some of these peptides have cancer-specific reactivity^{40;41}. A first-in-humans trial testing two of them has been initiated (NCT01846143). More data are needed to understand the extent of their cancer specificity and which among them may be true neoantigens.

Recent studies have highlighted the promise of targeting mutated neoantigens as cancer-rejection antigens, both as targets for tumor-infiltrating lymphocytes in adoptive T cell therapy, and as potential targets for cancer vaccines^{42–47}. This supports development of patient-specific personalized immune therapies, for which several clinical trials have been performed and are underway. However, there remain many unanswered questions about how best to induce immune responses to these antigens, as evidenced by the wide range of vaccine types and adjuvants employed in current and recent trials of vaccines using neoantigens (Table 3). Thus, work is needed to optimize cancer vaccine strategies and their adjuvants.

Forms of tumor antigen for use in cancer vaccines

Tumor antigens can be presented to the immune system as live attenuated tumor cells, killed tumor cells, tumor-lysate, protein, peptide, DNA, RNA, or other composite preparations⁴⁸. They can be administered with other immunologically active agents, pulsed on dendritic cells, or encoded in viral DNA. They can be administered as vaccines or they can be used to prime T cells for adoptive T cell transfer. These different forms of cancer antigens are discussed briefly below:

Peptide vaccines

are easily synthesized and stored and their use has been well-tolerated in numerous clinical trials. They continue to be investigated in vaccine trials for a range of cancers^{48;49}. The peptides may represent minimal T cell epitopes or longer peptides. They may also be given as single peptides or as mixtures of multiple peptides.

Minimal epitope peptides for CD8 T cells are typically 9 amino-acids in length. They have induced immune response rates of up to 100%⁹. These peptides directly bind to Class I MHC (MHC I) molecules on the surface of immature CTL, thereby circumventing antigen processing and directly activating effector cells. However, short peptides can also bind to MHC I on cells other than professional antigen presenting cells (eg fibroblasts in skin); since those cells lack costimulatory molecules CD80 and CD86, this effect may induce tolerance. Nonetheless, minimal epitope peptides can induce peptide-specific CD8⁺T cell responses in up to 100% of treated patients. These responses may comprise up to 1–5% of circulating CD8⁺T-cells^{9;10;50}. They can be used as a single peptide or as combinations of many⁵¹. The most common side effects have been low grade erythema or induration at the injection site, proteinuria and hypertension⁴⁹. In a trial for a mixture of 20 peptides, no grade 3 or 4 treatment related adverse events occurred in 17 patients⁵². Limitations to vaccines using these short peptides include a) rapid degradation by serum and tissue peptidases⁵³, b) transient immune responses of variable magnitude⁵⁴, c) applicability limited by MHC class I allele expression and d) limited antigen variety.

Long peptides are designed to include minimal epitopes for T cells but to be longer (typically 20–30 amino acids) requiring internalization in professional antigen presenting cells (e.g. dendritic cells) for processing and presentation of the MHC-restricted antigens^{55;56}. This avoids presentation by toleragenic cells lacking costimulatory molecules. Clinical experience with long peptide vaccines have included a pilot study with 4 long NY-ESO-1 peptides, which induced CD8⁺ and CD4⁺ T cell responses and antibody to the peptides, and a trial with long HPV E6 peptides that induced T cell responses and a high rate of complete regression of HPV-associated vulvar neoplasia. Long peptides also may contain more epitopes, enabling presentation across a large number of MHC alleles⁵⁷. On the other hand, the length of these peptides can include a number of self-epitopes, thereby predisposing the host to additional auto-reactivity.

Helper peptides: CD4⁺T helper cells play important roles in activating dendritic cells, in T cell memory, and in supporting CD8⁺ T cell responses. They are activated by recognition of cognate peptide antigens restricted by class II MHC molecules. A clinical trial with a mixture of 6 peptide antigens recognized by melanoma-reactive CD4⁺ T cells (6 melanoma helper peptides, 6MHP) induced Th1-dominant CD4⁺ T cell responses in 81% of patients, epitope spreading to CD8⁺ T cell responses in 45% of patients evaluated, and durable objective clinical responses and stable disease lasting 1–7 years in a subset of patients^{58–60}. In follow-up trials, those helper peptides did not increase the CD8⁺ T cell responses to Class I MHC restricted peptides, but in patients vaccinated with 6MHP, there was a strong and significant association between immune response to the melanoma helper peptides and

survival^{61;62}. Thus, vaccination with helper peptides offers therapeutic promise, perhaps in combination with other immune therapies.

DNA/RNA vaccines and viral or bacterial vectors

DNA and RNA vaccines rely on tumor antigen presentation by dendritic cells and myocytes in which the DNA or RNA is introduced^{63;64}. These vaccines are introduced either as naked DNA or RNA expressing tumor antigens or through a viral or bacterial vector. Delivery can be via an intramuscular, subcutaneous or oral route. Genes encoding immune enhancers like GM-CSF, IL-2, or heat shock proteins can also be included⁶⁵. DNA vaccines are easy to synthesize and store, and their safety has been proven in many clinical trials. A DNA vaccine encoding tyrosinase has been approved for veterinary use in dogs⁶⁶. However, efficacy was only seen in a fraction of patients⁶³. New approaches may improve immune responses induced by DNA vaccines⁶⁷. Recently, studies have shown that *Listeria monocytogenes* encoding tumor antigens and GM-CSF has the ability to selectively infect APCs and activate antigen presentation, with potential promise as a DNA vector for tumor vaccination in preclinical studies and animal trials⁶⁸. A clinical trial with a *Listeria* vaccine in pancreatic cancer has shown promise¹⁴ and is being tested further (NCT02004262).

Tumor cells lysate and whole tumor cell preparations

present a complete spectrum of antigens within the tumor cells. Thus, these are appealing, especially when the relevant tumor antigens are not directly identified. On the other hand, tumor cells contain numerous normal self proteins as well, which have the potential to induce autoimmunity⁶⁹. A small randomized trial of a vaccine incorporating secreted antigens secreted from melanoma cell lines suggested clinical benefit⁷⁰ and has led to an ongoing phase III trial (NCT01546571). On the other hand, several clinical trials of whole cell vaccines have failed to improve clinical outcome in phase III clinical trials, but provocative data in a subgroup analysis for a tumor cell lysate vaccine leave open the potential for benefit with this approach^{71–73}. Autologous tumor vaccines are more complex to prepare than allogeneic vaccines, but offer the potential benefit of including unique mutated neoantigens that may be important tumor rejection antigens. Thus, there is rationale for autologous tumor vaccine approaches.

Oncolytic viruses, intralesional therapy, and in situ vaccination

Pathogens injected directly into tumors create an inflammatory reaction that may promote immune cell infiltration in an otherwise immunosuppressed tumor micro-environment. Introducing lytic viruses also adds the ability to kill malignant cells directly, and ideally results in immunogenic cell death of those malignant cells. Oncolytic vaccine therapy relies on these concepts; it consists of in situ administration of genetically engineered viruses that target tumor cells while sparing host tissue. The destruction of cancer cells within a local inflammatory environment sets the stage for the adaptive immunity to recognize tumor specific antigens and to establish memory to combat disease progression. In 2005, the first oncolytic virus (H101, a mutated adenovirus) was approved in China to be used with Cisplatin and 5-FU for treatment of advanced nasopharyngeal carcinoma⁷⁴.

Insertion of genes into oncolytic viruses can result in specific overexpression in target cells. Genes coding for toll-like receptors (TLRs), heat shock proteins (HSPs), GM-CSF and tumor antigens have been introduced to enhance antitumor immunity and to overcome the immunosuppressive barriers of the tumor microenvironment⁷⁵. Recently, a phase 3 trial (OPTiM) of talimogene laherparepvec (T-VEC), a genetically engineered oncolytic herpes simplex virus expressing GM-CSF, resulted in an objective response rate of 26% and a complete response rate of 11% in stage IIIB/C and IV melanoma patients with cutaneous, subcutaneous or nodal disease¹⁹. Compared to a control group treated with systemic GM-CSF only, there was significant improvement in progression free survival and in objective response rate. Preliminary data from a clinical trial of T-VEC in combination with ipilimumab has also shown promise, with a 41% objective response rate, including 24% complete responses⁷⁶. Multiple other oncolytic viruses are also promising and are active in clinical trials, alone or in combination with other therapies. Other intralesional therapies also hold promise, with some showing abscopal effects, presumably due to inducing antitumor immunity from in situ vaccination^{77;78}. These intralesional therapies have employed toll-like receptor agonists, cytokines, checkpoint blockade, radiation therapy, and other agents, with encouraging outcomes for some patients^{77–79}.

Antigen-pulsed dendritic cells (DC)

For all cancer vaccines, immune responses depend on presentation of the vaccine antigens by dendritic cells. They may be activated in situ when a vaccine is administered with adjuvants that activate DC. Another common approach is to prepare DC populations ex vivo, usually from peripheral blood monocytes, and to pulse the DC with antigen ex vivo, with or without an additional maturation stimulus. These DC may be administered intradermally, subcutaneously, intramuscularly, intranodally, or intravenously. The route of vaccination with DC vaccines may define, in part, the target tissues to which the resulting T cells home. Several DC vaccine approaches have induced promising immune responses and clinical outcomes. However, two clinical trials have randomized patients to vaccination either with peptides administered with adjuvant (incomplete Freund's adjuvant) or peptides pulsed on dendritic cells; in both of these studies, the immune responses were better with peptides in adjuvant rather than the DC vaccine^{80;81}.

Sipuleucel-T, a DC vaccine, was the first antitumor vaccine to be approved by the FDA in 2011 following a phase III trial that showed an overall survival improvement of 4.1 months in select prostate cancer patients⁸. Sipuleucel-T is prepared from a patient's own (autologous) peripheral blood mononuclear cells isolated by leukapheresis, then shipped to a central facility, where the cell preparation is enriched for DC by gradient centrifugation. The DC are then co-cultured 36–44 hours with PA2024, a recombinant fusion protein comprising prostatic acid phosphatase (PAP) and granulocyte-macrophage colony stimulating factor (GM-CSF). The cells are then harvested and shipped to the patient's treatment facility for intravenous infusion^{8;27}.

Vaccine adjuvants

Adjuvants administered locally at the site of vaccination can have several functions to augment immune responses: (1) to activate innate immunity, (2) to steer the immune

response toward Th1 or Th2 immunity, and (3) to serve as a local depot for continued antigen release and/or protection from degradation^{82;83}. Other agents may be administered systemically to support T cell expansion or to reduce tumor associated immune dysfunction, and these may be considered systemic adjuvants.

Many peptide and protein vaccines have been administered in an emulsion with an incomplete Freund's adjuvant (IFA). IFA has long been an effective adjuvant for inducing antibody responses to proteins⁸⁴ and it is currently used in veterinary vaccines. Peptide vaccines using IFA have been immunogenic, but some of the immune responses are transient, and some are of low magnitude. Recent data identify potential negative effects of IFA vaccines: in a murine model, vaccination with short peptides in IFA induces preferential homing of antigen-specific T cells to the vaccine site, where they are sequestered and die⁸⁵. This limits T cell homing to the tumor. A study in humans corroborated this finding, with CD8+ T cells retained at sites of vaccination at least 6 weeks after the last vaccine, and T cells appearing dysfunctional^{86;87}. In murine studies, improved tumor control and more durable T cell responses can be obtained either by vaccinating with short peptides in an adjuvant comprised of a TLR agonist and an agonistic CD40 antibody, or by vaccinating with a long (20-mer) peptide in IFA⁸⁵. Thus, there appear to be complex interactions between the form of antigen and the optimal type of adjuvant.

Historically, a live mycobacterial product, Bacille Calmette-Guerin, BCG, has been used as a vaccine adjuvant and for intratumoral therapy. Unfortunately, BCG carries risk of systemic mycobacterial infection, which limits its use; though it is approved for use in topical treatment of superficial bladder cancer. Beneficial effects of BCG likely are mediated in part by activation of TLR. Other TLR agonists are being studied as vaccine adjuvants, as they can directly activate DC and have shown promise as vaccine adjuvants. The TLR3 agonist polyICLC and TLR9 agonist significantly increase CD8 and/or CD4⁺ T cell responses to peptide vaccines when combined with IFA. TLR1/2 agonists covalently bound to long peptides are also effective, and other TLR agonists show promise. New data highlight the roles for the STING (stimulators of interferon genes) pathway in activating innate immunity, and cyclic dinucleotides functioning as STING agonists are being investigated as adjuvants for cancer vaccines and for intratumoral use to create in situ vaccines⁸⁸.

Cytokines have promise as vaccine adjuvants, with some preclinical and clinical data supporting use of interleukin-12 (IL-12) locally⁸⁹ but conflicting data on the value of GM-CSF as a vaccine adjuvant⁹⁰. In murine studies, GM-CSF was a very effective adjuvant for whole tumor-cell vaccines and also increased T cell responses to peptide vaccines^{91;92}. However, in two randomized trials, GM-CSF had significant negative effects, when added to IFA or with BCG, either for immune response or for patient survival. On the other hand, GM-CSF given systemically with checkpoint blockade may have clinical benefit⁹³. Unfortunately, most human trials with GM-CSF have not been randomized, so its role in the clinical activity of T-vec and other immune therapies remains to be clarified.

Other vaccine adjuvants may include systemic agents to diminish immune dysfunction. Lowdose cyclophosphamide has been proposed as an agent to decrease regulatory T cells, with promising data in one clinical trial²⁰ but without clear benefit in other studies^{61;94}.

Numerous other approaches to activate innate immunity and to support T cell expansion offer promise as vaccine adjuvants, including an agonistic antibody to CD40 which was effective in conjunction with TLR agonists in murine models and but remains to be tested adequately in clinical trials⁹⁵.

Challenges in optimizing cancer vaccines

As detailed above, there is new promise for cancer vaccines; several approaches have induced promising clinical impact in phase II trials. However, it is important to acknowledge that multiple prior trials of cancer vaccines have failed to improve survival in phase III trials. In at least two cases, there was even a trend to worse outcome with vaccines^{73;96} although in others there was a trend toward better outcome⁹⁷. The reasons for those failed trials are not fully elucidated, but may relate to inadequate immunogenicity, short duration of immune responses, and failure to elicit both CD4⁺ and CD8⁺ T cell responses. Several vaccine adjuvants may interfere rather than improve T cell responses to vaccines^{50;85;87;98}. Thus, there is a need to optimize adjuvants for each form of antigen, which will require a better understanding of the effects of those adjuvants locally and regionally. Another major challenge to the effectiveness of cancer vaccines in patients with advanced cancer is the common failure of vaccine-induced T cells to infiltrate tumor deposits. These challenges are highlighted in the cancer:immunity cycle discussed by Chen and Mellman⁹⁹. Melanoma metastases represent immunologic sites that resist the immunotherapeutic effects of systemic therapies¹⁰⁰. Less than 10% of melanoma metastases have diffuse T cell infiltrates (Immunotype C)³; it is reasonable to hypothesize that if T cells fail to transmigrate intratumoral endothelium (Immunotype A) or fail to infiltrate tumor cell nests (Immunotype B), then circulating T cells may fail to infiltrate that majority of tumors. An interesting approach is to identify patients with favorable immunologic features in their tumors. The MAGE-A3 vaccine trial for melanoma failed to meet its first primary clinical endpoint of overall survival, but is still open for assessment of whether patients with favorable immune signatures in their tumors may in fact benefit from that vaccine^{28;101}. Regardless of the result of that trial, there may well be value in selecting patients for vaccines based on their tumor microenvironment. For patients with advanced cancer it may prove beneficial, to combine either systemic or intratumoral therapy with vaccines to increase the ability of vaccine-induced T-cells to infiltrate the malignant tumor cell nests and to mediate tumor control in a larger proportion of patients.

Conclusion

Immune therapy of cancer has long focused on melanoma but new data with adoptive T cell transfer and with checkpoint blockade therapy identified many cancers as responsive to immune therapy. The toxicities and costs of these latter approaches are significant and may limit applicability across the whole patient population. A goal of cancer vaccines, alone or in combination with other immune modulators, is to induce protective or therapeutic antitumor immunity that may mimic the benefits of current active immune therapies but with lower toxicity and cost. There remain many questions about how to optimize cancer vaccines in terms of the antigen selection, antigen form, vaccine adjuvants, and choices of combinations. With emerging access to next generation sequencing and specialized

laboratories for cellular therapy, personalized treatments will cost less and can become more feasible. Continuing discoveries of neoantigens and optimization of antigen formulations may also identify target antigens critical to tumor survival or unique to tumor cells. These will all factor into future approaches to cancer vaccines.

Acknowledgments

This work has been supported in part by T32 CA163177 (JO, YH) and U01 CA178846 (CLS)

Reference List

1. Denkert C, von MG, Brase JC, et al. Tumor-Infiltrating Lymphocytes and Response to Neoadjuvant Chemotherapy With or Without Carboplatin in Human Epidermal Growth Factor Receptor 2-Positive and Triple-Negative Primary Breast Cancers. *J Clin Oncol*. 2014 JCO.
2. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*. 2006; 313:1960–1964. [PubMed: 17008531]
3. Erdag G, Schaefer JT, Smolkin ME, et al. Immunotype and immunohistologic characteristics of tumor infiltrating immune cells are associated with clinical outcome in metastatic melanoma. *Cancer Res*. 2012; 72:1070–1080. [PubMed: 22266112]
4. Clarke B, Tinker AV, Lee CH, et al. Intraepithelial T cells and prognosis in ovarian carcinoma: novel associations with stage, tumor type, and BRCA1 loss. *Mod Pathol*. 2009; 22:393–402. [PubMed: 19060844]
5. Agarwalla P, Barnard Z, Fecci P, Dranoff G, Curry WT Jr. Sequential immunotherapy by vaccination with GM-CSF-expressing glioma cells and CTLA-4 blockade effectively treats established murine intracranial tumors. *J Immunother*. 2012; 35:385–389. [PubMed: 22576343]
6. Ly LV, Sluijter M, Versluis M, et al. Peptide vaccination after T-cell transfer causes massive clonal expansion, tumor eradication, and manageable cytokine storm. *Cancer Res*. 2010; 70:8339–8346. [PubMed: 20940397]
7. Lou Y, Wang G, Lizée G, et al. Dendritic cells strongly boost the antitumor activity of adoptively transferred T cells in vivo. *Cancer Res*. 2004; 64:6783–6790. [PubMed: 15374997]
8. Higano CS, Schellhammer PF, Small EJ, et al. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. *Cancer*. 2009; 115:3670–3679. [PubMed: 19536890]
9. Slingluff CL Jr, Petroni GR, Chianese-Bullock KA, et al. Immunologic and clinical outcomes of a randomized phase II trial of two multi-peptide vaccines for melanoma in the adjuvant setting. *Clin Cancer Res*. 2007; 13:6386–6395. [PubMed: 17975151]
10. Rosenberg SA, Sherry RM, Morton KE, et al. Tumor progression can occur despite the induction of very high levels of self/tumor antigen-specific CD8+ T cells in patients with melanoma. *J Clin Oncol*. 2005; 23:16169–16176.
11. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med*. 2004; 10:909–915. [PubMed: 15340416]
12. Schwartzentruber DJ, Lawson DH, Richards JM, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. *N Engl J Med*. 2011; 364:2119–2127. [PubMed: 21631324]
13. Kenter GG, Welters MJ, Valentijn AR, et al. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. *N Engl J Med*. 2009; 361:1838–1847. [PubMed: 19890126]
14. Le DT, Wang-Gillam A, Picozzi V, et al. Safety and Survival With GVAX Pancreas Prime and Listeria Monocytogenes-Expressing Mesothelin (CRS-207) Boost Vaccines for Metastatic Pancreatic Cancer. *J Clin Oncol*. 2015 JCO.
15. Swartz AM, Li QJ, Sampson JH. Rindopepimut: a promising immunotherapeutic for the treatment of glioblastoma multiforme. *Immunotherapy*. 2014; 6:679–690. [PubMed: 25186601]
16. Schuster J, Lai RK, Recht LD, et al. A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study. *Neuro Oncol*. 2015 nov348.

17. Mittendorf EA, Clifton GT, Holmes JP, et al. Clinical trial results of the HER-2/neu (E75) vaccine to prevent breast cancer recurrence in high-risk patients: from US Military Cancer Institute Clinical Trials Group Study I-01 and I-02. *Cancer*. 2012; 118:2594–2602. [PubMed: 21989902]
18. Carmichael MG, Benavides LC, Holmes JP, et al. Results of the first phase 1 clinical trial of the HER-2/neu peptide (GP2) vaccine in disease-free breast cancer patients: United States Military Cancer Institute Clinical Trials Group Study I-04. *Cancer*. 2010; 116:292–301. [PubMed: 19924797]
19. Kaufman HL, Bines SD. OPTIM trial: a Phase III trial of an oncolytic herpes virus encoding GM-CSF for unresectable stage III or IV melanoma. *Future Oncol*. 2010; 6:941–949. [PubMed: 20528232]
20. Walter S, Weinschenk T, Reinhardt C, Singh-Jasuja H. Single-dose cyclophosphamide synergizes with immune responses to the renal cell cancer vaccine IMA901. *Oncoimmunology*. 2013; 2:e22246. [PubMed: 23482454]
21. Walter SWT, Stenzl A. Multipetide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. *Nat Med*. 2012 published online July 2012.
22. GlaxoSmithKline. [4-2-2014. 1-4-2015] Update on phase III clinical trial of investigational MAGE-A3 antigen-specific cancer immunotherapeutic in non-small cell lung cancer. <http://us.gsk.com/en-us/media/press-releases/2014/update-on-phase-iii-clinical-trial-of-investigational-mage-a3-antigen-specific-cancer-immunotherapeutic-in-non-small-cell-lung-cancer/>
23. Kruit WH, Suci S, Dreno B, et al. Selection of immunostimulant AS15 for active immunization with MAGE-A3 protein: results of a randomized phase II study of the European Organisation for Research and Treatment of Cancer Melanoma Group in Metastatic Melanoma. *J Clin Oncol*. 2013; 31:2413–2420. [PubMed: 23715572]
24. Kruit WH, Suci S, Dreno B, et al. Immunization with recombinant MAGE-A3 protein combined with adjuvant systems AS15 or AS02B in patients with unresectable and progressive metastatic cutaneous melanoma: A randomized open-label phase II study of the EORTC Melanoma Group (16032-18031). *J Clin Oncol*. 2008; 26 abstract 9065.
25. Louahed J, Gruselle O, Gaulis S, et al. Expression of defined genes identified by pretreatment tumor profiling: Association with clinical responses to GSK MAGE-A3 immunotherapeutic in metastatic melanoma patients (16032-18031). [abstract] Louahed J, Gruselle O, Gaulis S, et al. *J Clin Oncol*. 2008; 26 Abstract 9045.
26. Gulley JL, Madan RA, Tsang KY, et al. Immune impact induced by PROSTVAC (PSATRICOM), a therapeutic vaccine for prostate cancer. *Cancer Immunol Res*. 2014; 2:133–141. [PubMed: 24778277]
27. Kantoff PW, Schuetz TJ, Blumenstein BA, et al. Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. *J Clin Oncol*. 2010; 28:1099–1105. [PubMed: 20100959]
28. Glaxo Smith Kline. The investigational MAGE-A3 antigen-specific cancer immunotherapeutic does not meet first co-primary endpoint in Phase III melanoma clinical trial. <http://www.gsk.com/en-gb/media/press-releases/2013/the-investigational-mage-a3-antigen-specific-cancer-immunotherapeutic-does-not-meet-first-co-primary-endpoint-in-phase-iii-melanoma-clinical-trial/>.
29. Johnson LA, Morgan RA, Dudley ME, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. *Blood*. 2009; 114:535–546. [PubMed: 19451549]
30. Beatty GL, Haas AR, Maus MV, et al. Mesothelin-specific Chimeric Antigen Receptor mRNA-Engineered T cells Induce Anti-Tumor Activity in Solid Malignancies. *Cancer Immunol Res*. 2014; 2:112–120. [PubMed: 24579088]
31. Henderson RA, Konitsky WM, Barratt-Boyes SM, Soares M, Robbins PD, Finn OJ. Retroviral expression of MUC-1 human tumor antigen with intact repeat structure and capacity to elicit immunity in vivo. *Journal of Immunotherapy*. 1998; 21:247–256. [PubMed: 9672846]
32. Siroy AE, Boland GM, Milton DR, et al. Beyond BRAF(V600): clinical mutation panel testing by next-generation sequencing in advanced melanoma. *J Invest Dermatol*. 2015; 135:508–515. [PubMed: 25148578]

33. Fritsch EF, Rajasagi M, Ott PA, Brusic V, Hacohe N, Wu CJ. HLA-binding properties of tumor neoepitopes in humans. *Cancer Immunol Res*. 2014; 2:522–529. [PubMed: 24894089]
34. Heestand GM, Kurzrock R. Molecular landscape of pancreatic cancer: implications for current clinical trials. *Oncotarget*. 2015
35. Robbins PF, el-Gamil M, Li YF, Fitzgerald EB, Kawakami Y, Rosenberg SA. The intronic region of an incompletely spliced gp100 gene transcript encodes an epitope recognized by melanoma-reactive tumor-infiltrating lymphocytes. *J. 1997; 159:303–308*.
36. Fijak M, Bhushan S, Meinhardt A. Immunoprivileged sites: the testis. *Methods Mol Biol*. 2011; 677:459–70. [PubMed: 20941627]
37. Gjerstorff MF, Burns J, Ditzel HJ. Cancer-germline antigen vaccines and epigenetic enhancers: future strategies for cancer treatment. *Expert Opin Biol Ther*. 2010; 10:1061–1075. [PubMed: 20420535]
38. Robbins PF, Morgan RA, Feldman SA, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J Clin Oncol*. 2011; 29:917–924. [PubMed: 21282551]
39. Morgan RA, Chinnasamy N, Abate-Daga D, et al. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. *J Immunother*. 2013; 36:133–151. [PubMed: 23377668]
40. Cobbold M, De La Pena H, Norris A, et al. MHC Class I-Associated Phosphopeptides Are the Targets of Memory-like Immunity in Leukemia. *Sci Transl Med*. 2013; 5:203ra125.
41. Depontieu FR, Qian J, Zarling AL, et al. Identification of tumor-associated, MHC class II-restricted phosphopeptides as targets for immunotherapy. *Proc Natl Acad Sci U S A*. 2009; 106:12073–12078. [PubMed: 19581576]
42. Lu YC, Yao X, Crystal JS, et al. Efficient identification of mutated cancer antigens recognized by T cells associated with durable tumor regressions. *Clin Cancer Res*. 2014; 20:3401–3410. [PubMed: 24987109]
43. Lu YC, Yao X, Li YF, et al. Mutated PPP1R3B is recognized by T cells used to treat a melanoma patient who experienced a durable complete tumor regression. *J Immunol*. 2013; 190:6034–6042. [PubMed: 23690473]
44. Charen AS, Makarov V, Merghoub T, et al. The neoantigen landscape underlying clinical response to ipilimumab [abstract] Charen AS, Makarov V, Merghoub T et al. *J Clin Oncol (Meeting Abstracts)*. 2014; 32 Abstract 3003.
45. van Rooij N, van Buuren MM, Philips D, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. *J Clin Oncol*. 2013; 31:e439–e442. [PubMed: 24043743]
46. Zorn E, Hercend T. A natural cytotoxic T cell response in a spontaneously regressing human melanoma targets a neoantigen resulting from a somatic point mutation. *Eur J Immunol*. 1999; 29:592–601. [PubMed: 10064075]
47. Matsushita H, Vesely MD, Koboldt DC, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. *Nature*. 2012; 482:400–404. [PubMed: 22318521]
48. Tagliamonte M, Petrizzo A, Ternesello ML, Buonaguro FM, Buonaguro L. Antigen-specific vaccines for cancer treatment. *Hum Vaccin Immunother*. 2014; 10:3332–3346. [PubMed: 25483639]
49. Aranda F, Vacchelli E, Eggermont A, et al. Trial Watch: Peptide vaccines in cancer therapy. *Oncoimmunology*. 2013; 2:e26621. [PubMed: 24498550]
50. Slingluff CL Jr, Petroni GR, Olson WC, et al. Effect of GM-CSF on circulating CD8+ and CD4+ T cell responses to a multi-peptide melanoma vaccine: Outcome of a multicenter randomized trial. *Clin Cancer Res*. 2009; 15:7036–7044. [PubMed: 19903780]
51. Slingluff CL Jr. The present and future of peptide vaccines for cancer: single or multiple, long or short, alone or in combination? *Cancer J*. 2011; 17:343–350. [PubMed: 21952285]
52. Noguchi M, Arai G, Matsumoto K, et al. Phase I trial of a cancer vaccine consisting of 20 mixed peptides in patients with castration-resistant prostate cancer: dose-related immune boosting and suppression. *Cancer Immunol Immunother*. 2015

53. Brinckerhoff LH, Kalashnikov VV, Thompson LW, et al. Terminal modifications inhibit proteolytic degradation of an immunogenic MART-1(27-35) peptide: implications for peptide vaccines. *Int J Cancer*. 1999; 83:326–334. [PubMed: 10495424]
54. Knutson KL, Schiffman K, Cheever MA, Disis ML. Immunization of cancer patients with a HER-2/neu, HLA-A2 peptide, p369-377, results in short-lived peptide-specific immunity. *Clin Cancer Res*. 2002; 8:1014–1018. [PubMed: 12006513]
55. Melief CJ, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. *Nat Rev Cancer*. 2008; 8:351–360. [PubMed: 18418403]
56. Bijker MS, van den Eeden SJ, Franken KL, Melief CJ, van der Burg SH, Offringa R. Superior induction of anti-tumor CTL immunity by extended peptide vaccines involves prolonged, DC-focused antigen presentation. *Eur J Immunol*. 2008; 38:1033–1042. [PubMed: 18350546]
57. Sabbatini P, Tsuji T, Ferran L, et al. Phase I Trial of Overlapping Long Peptides from a Tumor Self-Antigen and Poly-ICLC Shows Rapid Induction of Integrated Immune Response in Ovarian Cancer Patients. *Clin Cancer Res*. 2012; 18:6497–6508. [PubMed: 23032745]
58. Dillon PM, Olson WC, Czarkowski A, et al. A melanoma helper peptide vaccine increases Th1 cytokine production by leukocytes in peripheral blood and immunized lymph nodes. *J Immunother Cancer*. 2014; 2:23. eCollection; 2014.:23-2. [PubMed: 25126421]
59. Slingluff CL Jr, Petroni GR, Olson W, et al. Helper T cell responses and clinical activity of a melanoma vaccine with multiple peptides from MAGE and melanocytic differentiation antigens. *J Clin Oncol*. 2008; 26:4973–4980. [PubMed: 18809608]
60. Hu Y, Petroni GR, Olson WC, et al. Immunologic hierarchy, class II MHC promiscuity, and epitope spreading of a melanoma helper peptide vaccine. *Cancer Immunol Immunother*. 2014
61. Slingluff CL Jr, Petroni GR, Chianese-Bullock KA, et al. A randomized multicenter trial of the effects of melanoma-associated helper peptides and cyclophosphamide on the immunogenicity of a multi-peptide melanoma vaccine. *J Clin Oncol*. 2011; 29:2924–2932. [PubMed: 21690475]
62. Slingluff CL Jr, Lee S, Zhao F, et al. A randomized phase II trial of multi-epitope vaccination with melanoma peptides for cytotoxic T-cells and helper T-cells for patients with metastatic melanoma (E1602). *Clin Cancer Res*. 2013; 19:4228–4238. [PubMed: 23653149]
63. Senovilla L, Vacchelli E, Garcia P, et al. Trial watch: DNA vaccines for cancer therapy. *Oncoimmunology*. 2013; 2:e23803. [PubMed: 23734328]
64. Boisguerin V, Castle JC, Loewer M, et al. Translation of genomics-guided RNA-based personalised cancer vaccines: towards the bedside. *Br J Cancer*. 2014; 111:1469–1475. [PubMed: 25314223]
65. Chen CH, Wang TL, Hung CF, et al. Enhancement of DNA vaccine potency by linkage of antigen gene to an HSP70 gene. *Cancer Res*. 2000; 60:1035–1042. [PubMed: 10706121]
66. Grosenbaugh DA, Leard AT, Bergman PJ, et al. Safety and efficacy of a xenogeneic DNA vaccine encoding for human tyrosinase as adjunctive treatment for oral malignant melanoma in dogs following surgical excision of the primary tumor. *Am J Vet Res*. 2011; 72:1631–1638. [PubMed: 22126691]
67. van den Berg JH, Oosterhuis K, Schumacher TN, Haanen JB, Bins AD. Intradermal vaccination by DNA tattooing. *Methods Mol Biol*. 2014; 1143:131–140. [PubMed: 24715286]
68. Wood LM, Paterson Y. Attenuated *Listeria monocytogenes*: a powerful and versatile vector for the future of tumor immunotherapy. *Front Cell Infect Microbiol*. 2014; 4:51. eCollection; 2014.:51. [PubMed: 24860789]
69. Ozao-Choy J, Lee DJ, Faries MB. Melanoma vaccines: mixed past, promising future. *Surg Clin North Am*. 2014; 94:1017–1030. viii. [PubMed: 25245965]
70. Bystryjn JC, Zeleniuch-Jacquotte A, Oratz R, Shapiro RL, Harris MN, Roses DF. Double-blind trial of a polyvalent, shed-antigen, melanoma vaccine. [see comment]. *Clin Cancer Res*. 2001; 7:1882–1887. [PubMed: 11448900]
71. Testori A, Richards J, Whitman E, et al. Phase III comparison of vitespen, an autologous tumor-derived heat shock protein gp96 peptide complex vaccine, with physician's choice of treatment for stage IV melanoma: the C-100-21 Study Group. *J Clin Oncol*. 2008; 26:955–962.

72. Sosman JA, Unger JM, Liu PY, et al. Adjuvant immunotherapy of resected, intermediate-thickness, node-negative melanoma with an allogeneic tumor vaccine: impact of HLA class I antigen expression on outcome. *J Clin Oncol.* 2002; 20:2067–2075. [PubMed: 11956267]
73. Morton DL, Mozzillo N, Thompson JF, et al. An international, randomized, phase III trial of bacillus Calmette-Guerin (BCG) plus allogeneic melanoma vaccine (MCV) or placebo after complete resection of melanoma metastatic to regional or distant sites. [abstract] Morton DL, Mozzillo N, Thompson JF et al. *Journal of Clinical Oncology*, 2007 ASCO Annual Meeting Proceedings Part I. 2007; 25 abstract 8508.
74. Woller N, Gurlevik E, Ureche CI, Schumacher A, Kuhnel F. Oncolytic viruses as anticancer vaccines. *Front Oncol.* 2014; 4:188. eCollection; 2014.:188. [PubMed: 25101244]
75. Elsedawy NB, Russell SJ. Oncolytic vaccines. *Expert Rev Vaccines.* 2013; 12:1155–1172. [PubMed: 24124877]
76. Puzanov I, Milhem M, Andtbacka RH, et al. Primary analysis of a phase 1b multicenter trial to evaluate safety and efficacy of talimogene laherparepvec (T-VEC) and ipilimumab (ipi) in previously untreated, unresected stage IIIB-IV melanoma [abstract] Puzanov I, Milhem M, Andtbacka RH et al. *J Clin Oncol.* 2014; 32 Abstract 9029.
77. Kim YH, Gratzinger D, Harrison C, et al. In situ vaccination against mycosis fungoides by intratumoral injection of a TLR9 agonist combined with radiation: a phase 1/2 study. *Blood.* 2012; 119:355–363. [PubMed: 22045986]
78. Goldstein MJ, Varghese B, Brody JD, et al. A CpG-loaded tumor cell vaccine induces antitumor CD4+ T cells that are effective in adoptive therapy for large and established tumors. *Blood.* 2011; 117:118–127. [PubMed: 20876455]
79. Salazar AM, Erlich RB, Mark A, Bhardwaj N, Herberman RB. Therapeutic in situ autovaccination against solid cancers with intratumoral poly-ICLC: case report, hypothesis, and clinical trial. *Cancer Immunol Res.* 2014; 2:720–724. [PubMed: 24801836]
80. Slingluff CL Jr, Petroni GR, Yamshchikov GV, et al. Clinical and immunologic results of a randomized phase II trial of vaccination using four melanoma peptides either administered in granulocyte-macrophage colony-stimulating factor in adjuvant or pulsed on dendritic cells. *J Clin Oncol.* 2003; 21:4016–4026. [PubMed: 14581425]
81. O'Neill DW, Adams S, Goldberg JD, et al. Comparison of the immunogenicity of Montanide ISA 51 adjuvant and cytokine-matured dendritic cells in a randomized controlled clinical trial of melanoma vaccines [abstract] O'Neill DW, Adams S, Goldberg JD et al. *J Clin Oncol (Meeting Abstracts).* 2009; 27 abstract 3002.
82. Lim YT. Vaccine adjuvant materials for cancer immunotherapy and control of infectious disease. *Clin Exp Vaccine Res.* 2015; 4:54–58. [PubMed: 25648865]
83. Marciani DJ. Vaccine adjuvants: role and mechanisms of action in vaccine immunogenicity. *Drug Discov Today.* 2003; 8:934–943. [PubMed: 14554157]
84. Freund J, Thomson KJ. A simple, rapid technic of preparing water-in-oil emulsions of penicillin, drugs and biologics. *Science.* 1945; 101:468–469. [PubMed: 17799002]
85. Hailemichael Y, Dai Z, Jaffarizad N, et al. Persistent antigen at vaccination sites induces tumor-specific CD8(+) T cell sequestration, dysfunction and deletion. *Nat Med.* 2013; 19:465–472. [PubMed: 23455713]
86. Schaefer JT, Patterson JW, Deacon DH, et al. Dynamic changes in cellular infiltrates with repeated cutaneous vaccination: A histologic and immunophenotypic analysis. *Journal of Translational Medicine.* 2010; 8:79. [PubMed: 20727190]
87. Salerno EP, Shea SM, Olson WC, et al. Activation, dysfunction and retention of T cells in vaccine sites after injection of incomplete Freund's adjuvant, with or without peptide. *Cancer Immunol Immunother.* 2013; 62:1149–1159. [PubMed: 23657629]
88. Dubensky TW Jr, Kanne DB, Leong ML. Rationale, progress and development of vaccines utilizing STING-activating cyclic dinucleotide adjuvants. *Ther Adv Vaccines.* 2013; 1:131–143. [PubMed: 24757520]
89. Lee P, Wang F, Kuniyoshi J, et al. Effects of interleukin-12 on the immune response to a multipptide vaccine for resected metastatic melanoma. *J Clin Oncol.* 2001; 19:3836–3847. [PubMed: 11559721]

90. Kaufman HL, Ruby CE, Hughes T, Slingluff CL Jr. Current status of granulocyte-macrophage colony-stimulating factor in the immunotherapy of melanoma. *J Immunother Cancer*. 2014; 2:11. eCollection;2014.:11-12. [PubMed: 24971166]
91. Dranoff G, Jaffee E, Lazenby A, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci USA*. 1993; 90:3539–3543. [PubMed: 8097319]
92. Ahlers JD, Dunlop N, Alling DW, Nara PL, Berzofsky JA. Cytokine-in-adjuvant steering of the immune response phenotype to HIV-1 vaccine constructs: granulocyte-macrophage colony-stimulating factor and TNF-alpha synergize with IL-12 to enhance induction of cytotoxic T lymphocytes. *Jl*. 1997; 158:3947–3958.
93. Hodi FS, Lee S, McDermott DF, et al. Ipilimumab plus sargramostim vs ipilimumab alone for treatment of metastatic melanoma: a randomized clinical trial. *JAMA*. 2014; 312:1744–1753. [PubMed: 25369488]
94. Emens LA, Asquith JM, Leatherman JM, et al. Timed sequential treatment with cyclophosphamide, doxorubicin, and an allogeneic granulocyte-macrophage colonystimulating factor-secreting breast tumor vaccine: a chemotherapy dose-ranging factorial study of safety and immune activation. *J Clin Oncol*. 2009; 27:5911–5918. [PubMed: 19805669]
95. Barrios K, Celis E. TriVax-HPV: an improved peptide-based therapeutic vaccination strategy against human papillomavirus-induced cancers. *Cancer Immunol Immunother*. 2012; 61:1307–1317. [PubMed: 22527249]
96. Hodi FS, O'Day SJ, McDermott DF, et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. *N Engl J Med*. 2010; 363:711–723. [PubMed: 20525992]
97. Sondak VK, Liu PY, Tuthill RJ, et al. Adjuvant immunotherapy of resected, intermediate-thickness, node-negative melanoma with an allogeneic tumor vaccine: overall results of a randomized trial of the Southwest Oncology Group. *J Clin Oncol*. 2002; 20:2058–2066. [PubMed: 11956266]
98. Faries MB, Hsueh EC, Ye X, Hoban M, Morton DL. Effect of granulocyte/macrophage colony-stimulating factor on vaccination with an allogeneic whole-cell melanoma vaccine. *Clin Cancer Res*. 2009; 15:7029–7035. [PubMed: 19903777]
99. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity*. 2013; 39:1–10. [PubMed: 23890059]
100. Weiss GR, Grosh WW, Chianese-Bullock KA, et al. Molecular insights on the peripheral and intratumoral effects of systemic high-dose rIL-2 (aldesleukin) administration for the treatment of metastatic melanoma. *Clin Cancer Res*. 2011; 17:7440–7450. [PubMed: 21976537]
101. Ulloa-Montoya F, Louahed J, Dizier B, et al. Predictive gene signature in MAGE-A3 antigen-specific cancer immunotherapy. *J Clin Oncol*. 2013; 31:2388–2395. [PubMed: 23715562]
102. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med*. 2013; 368:1509–1518. [PubMed: 23527958]

Figure 1.

Cancer vaccine trials listed as open at ClinicalTrials.gov September 2014. The number of trials for each cancer type are shown in the bar graph. The number with each type of antigen presentation is shown in the inset table. RCC = renal cell carcinoma; CRC = colorectal cancer; HNSCC = head and neck squamous cell carcinoma.

Figure 2.
Types of cancer antigens, with examples for each.

Figure 3.

Adjuvants being used in clinical trials of cancer vaccines. Cancer vaccine trials listed as open at ClinicalTrials.gov (Sept 2014) were assessed for the adjuvants used, showing the number of trials with each **A)** local and **B)** systemic vaccine adjuvants. IFA = incomplete Freund's adjuvant; IL-12 = interleukin-12; CD40L = CD40 ligand; Cy = cyclophosphamide; IDO1 = indoleamine-2,3-dis=xygenase inhibitor; CT-011 and nivolumab are PD-1 antibodies.

Danger is represented by:

These have molecular features that distinguish them from our own cells:

Our immune systems have evolved to recognize them:

Figure 4.

Cancer vaccines commonly employ agents that induce “danger” by mimicking foreign pathogens.

Table 1

Cancer vaccines in late stage development

Vaccine name (NCI thesaurus code)	Tumor target	Antigen (Ag)	Antigen presentation method	Evidence for immune response	Clinical activity	Design of Phase 3 trial
Rindopepimut (C69076) ^{15;16}	Glioblastoma multiforme	Mutated EGFRvIII	14-mer peptide, conjugated to KLH	Increased Ab titer in 85%; elimination of EGFRvIII in 4/6 examined tumor samples ¹⁶	26% overall survival at 3 years in single arm phase II trial.	NCT01480479 (ACT IV); phase III (TMZ +/- vaccine with GM-CSF); ongoing, not recruiting.
Nelipepimut-S (C117985) ^{17;18}	Breast cancer	Her peptide E75	Administered with GM-CSF	CD8+ T cell responses in 100% of patients	DFS 90% for vaccinated patients vs 80% for concurrent non-randomized controls at 5 years	NCT01479244 (PRESENT); phase III (vaccine + GM-CSF vs. GM-CSF alone)
Talimogene Laherpaprepvec (C61314) ¹⁹	Melanoma	In situ vaccination with oncolytic virus	Oncolytic virus: Modified herpes virus encoding GM-CSF	Regression of metastases not injected with T-vec	ORR 26%; CRR 11% in stage IIIB/C and IV; improved outcome vs. GM-CSF alone.	NCT00769704 (OptiM) trial was a phase III trial that met its primary endpoint of improving durable response rate.
Renal cell carcinoma peptides vaccine IMA901 (C70968) ^{20;21}	Renal cell carcinoma	10 peptides of tumor associated peptides (TUMAPs)	Multipetide vaccine administered with GM-CSF and cyclophosphamide	T cell responses to TUMAPs observed in 20/27 evaluable patients	Immune responses to TUMAPs correlate with longer survival. Putative biomarkers identified.	NCT01265901; phase III sunitinib +/- vaccine
Live attenuated listeria encoding human mesothelin vaccine CRS-207 (C74055) ¹⁴	Pancreatic cancer	Mesothelin	Listeria monocytogenes engineered to express mesothelin; administered alone or with GVAX (allogeneic pancreatic cancer cells expressing GM-CSF)	Mesothelin-specific CD8 T cell response detected and associated with prolonged survival.	Median OS 6.1 months for CRS-207 and G-VAX/GM-CSF vs 3.9 months with GVAX+ GM-CSF only.	NCT02004262 (ECLIPSE); Phase IIB trial (CRS-2007 + GVAX vs. CRS-2007 alone vs chemotherapy)
Astuprotimut-R (C78478) ^{22-25;28}	Melanoma	MAGE-A3	recMAGE-A3 protein + AS15 immunostimulant.	High antibody titers; T cell induction	Objective responses in 4 patients (11%), including 2 durable CRs; median OS 33 months.	NCT00796445 (DERMA) phase III trial of vaccine vs placebo did not meet DFS primary endpoint. Co-primary endpoint of DFS in patients with immune

Vaccine name (NCI thesaurus code)	Tumor target	Antigen (Ag)	Antigen presentation method	Evidence for immune response	Clinical activity	Design of Phase 3 trial
	Lung CA	MAGE-A3	recMAGE-A3 protein + AS15	High antibody titers; T cell induction.	In phase II study, recurrence with vaccine was 35%, compared to 43% for placebo. HR for OS was 0.81 (NS)	signature is pending; signature is pending; NCT00480025 (MAGRIT) phase III trial of vaccine vs placebo did not meet clinical endpoints (DFS) and was terminated.
Fowlpox-PSA-TRICOM vaccine (C38708) ^{26,27}	Prostate CA	PSA protein encoded in virus	Fowlpox expressing Ag plus genes for 3 costimulatory molecules (TRICOM; B7.1, ICAM-1, LFA-3)	T cell response to PSA peptide increased in 57%; epitope spreading in 68%	In randomized phase II trial, no diff in PFS (primary endpt); but OS 30%, vs 17% in controls	NCT01322490; phase III (vaccine + GM-CSF; Vaccine + placebo; double placebo) closed to enrollment.
Synthetic long E6/E7 peptides vaccine HPV-01 (C111037) ¹³	HPV-induced neoplasms; e.g. vulvar neoplasia	HPV E6 and E7	Mixture of 13 long peptides (25–35 amino acids each) administered with adjuvant	CD4 & CD8 T cell responses detected & associated with CRs	Durable CRR 47% in advanced vulvar neoplasia	Not available

PSA = prostate-specific antigen; KLH = keyhole limpet hemocyanin; TMZ = temozolomide; ORR = objective response rate; CRR = complete response rate; HPV = human papillomavirus; GVAX = GM-CSF-expressing pancreatic cancer cell vaccine; DFS = disease-free survival; AS15 = CpG, monophosphoryl lipid A, QS-21.

Table 2

Tissue differentiation antigens and cancer-germ line antigens successfully targeted with cancer immunotherapy

Protein antigen	Tissue in which expressed	Cancer of this tissue	Form of antigen	Therapeutic immunotherapy targeting this antigen
CD19	B lymphocytes	B cell leukemias	Intact protein at cell surface	Rituximab (monoclonal antibody); CAR-T cells ¹⁰²
Prostatic acid phosphatase (PAP)	Prostate gland	Prostate cancer	PAP peptides presented by MHC	Sipuleucel-T (cellular vaccine) ⁸
gp100	Melanocytes	Melanoma	gp100 peptide presented by HLA-A2	Peptide vaccine combined with IL-2 (compared to IL-2 alone) ¹²
MART-1/MelanA	Melanocytes	Melanoma	MART-1 peptide presented by HLA-A2	T-cell receptor transduced T cells ²⁹
Mesothelin	Mesothelial cells (eg: pleura)	Pancreatic cancer, ovarian cancer, mesothelioma	Mesothelin peptide presented by HLA-A2	CAR-T cells ³⁰
NY-ESO-1	Germ line cells eg: testis	Multiple cancers, including synovial cell sarcoma	NY-ESO-1 peptide presented by HLA-A2	T-cell receptor transduced T cells ³⁸

Table 3Recent and active clinical trials of vaccines with mutated neoantigens (from [ClinicalTrials.gov](https://clinicaltrials.gov))

Trial ID	PI (Site) Country	Mutated neoantigens	Adjuvant
NCT00001703	Khleif (NIH) USA	mutated VHL peptides	Incomplete Freund's adjuvant
NCT00019006	Khleif (NCI) USA	mutated RAS peptides	DETOX-B
NCT00003959	Nimer (MSKCC) USA	mutated RAS peptides	GM-CSF
NCT00019331	Gause (Magnuson Ctr) USA	RAS peptides	GM-CSF, DETOX-PC, systemic interleukin-2
NCT01885702	de Vries (Radboud Univ) The Netherlands	Mutated neoantigens in MSI-high colorectal cancer and germline MMR-gene mutation	Dendritic cells
NCT01970358	Ott (DFCI) USA	personalized mutated neoantigens	polyICLC

MSKCC = Memorial Sloan Kettering Cancer Center; DFCI = Dana Farber Cancer Institute; GM-CSF = granulocyte-macrophage colony stimulating factor